stains were used to evaluate compartment size (H&E), ECM organization (picrosirius and cross-polar microscopy), immune infiltration and elastosis (orcein/giemsa), glycation (periodic acid Schiff) and new collagen synthesis (Herovici). Novel algorithmic methods facilitated quantitative histomorphometry. A systematic review of transcriptomic data was performed using guided and unguided bioinformatic methods to investigate mechanisms perturbed in ageing fibroblasts, particularly those deleterious to ECM. A subset of key processes was investigated *in vitro*.

Results From 3 months, dermal depth progressively decreased, accompanied by increased relaxation of collagen 'basket weave', with a loss of perpendicular fibres in extreme age. An increase in mature collagen fibres relative to young fibres also characterized ageing, and new collagen synthesis became more discontinuous. These observations were confirmed using novel image filtering and transformation techniques designed to isolate structural information. Increased glycation (especially basement membranes) was seen only in 20-month-old skin, as were elastosis and increased mast cell infiltration. Bioinformatic analysis revealed critical processes in fibroblast ageing, including alterations in matrix metalloproteases, cytokines and collagen biosynthesis, the last of which we confirmed in a passage-aged fibroblast model.

Discussion Ageing is a complex process, and we combined bioinformatic, histological, cell culture and imaging techniques to investigate this phenomenon in skin. Pathways isolated by bioinformatics were consistent with the loss of structure observed histologically, and we are pursuing these using *in vitro* models. An improved understanding of mechanisms driving compromised cutaneous integrity and function may ultimately yield new approaches to ameliorate damage and promote health.

A4

Dual roles of heparan sulphate in sonic hedgehog (Shh) release and processing

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Introduction All Hedgehog (Hh) proteins are released from producing cells, despite being synthesized as dually lipidated molecules (ShhNp), a modification that normally leads to firm tethering of proteins to the cell membrane. We found that proteolytic Sonic Hh 'ectodomain shedding', mediated by a disintegrin and metalloprotease family members 10, 12 (Ohlig *et al.* 2011) and 17 (Dierker *et al.* 2009), results in Hh solubilization. The observed redundancy in sheddase function raises the question of how ShhNp release is regulated. GPI-linked heparan sulphate proteoglycans (HSPGs) co-localize with Hhs; therefore, we investigated possible roles of HS(PGs) in Shh release and processing.

Materials and Methods In addition to the ADAMs, we tested matrix metalloproteases 2 and 9 in gain- and loss-of-function experiments for their ability to release Shh. To test whether GPI-linked glypicans can affect Shh release, we co-expressed ShhNp and glypican 6 (Glp6). To control for any potential function of the associated HS chains, we expressed HS-unmodified Glp6. Moreover, we confirmed HS-dependent processing of terminal ShhN peptides *in vitro* using tagged forms of the recombinantly produced morphogen. To test the alternative possibility that HS directly activates proteases involved in Shh processing, we overexpressed MMP2 and 9 in the presence or absence of specific forms of HS and analysed resulting MMP zymogen activation by gelatin zymography.

Results We provide evidence that Shh shedding can be mediated by MMP9 and that Glp6 co-expression regulates Shh release and processing, likely via the HS chains and direct modulation of sheddase activation and specificity. We further demonstrate direct and HS-sulphation-specific regulation of Shh processing in cell-free assays. Moreover, we observed that the presence of specific HS stabilized the processed morphogen in solution.

Discussion From these data, we suggest a model explaining ShhNp spreading in the ECM based on its initial lipidation and subsequent metalloprotease-mediated processing. HSPG interactions in this process would be required for the clustering on the producing cell, and specific forms of HS would regulate the activity of associated metalloproteases of the ADAM and MMP families, resulting in the release of processed Shh from the cell surface. Lastly, cell surface tethered but possibly also processed ShhNp is stabilized from degradation via interactions with HSPGs.

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A6

Gender modulates the molecular response to acute joint destabilization

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Introduction It is well established that gender is an independent risk factor for the development of osteoarthritis (OA). Postmenopausal women have an increase in OA that has been attributed to changes in sex hormones and led to the suggestion that oestrogen is chondroprotective. This is supported by the observation that the disease severity of surgically induced OA in female premenopausal mice is significantly lower than in males and that in female mice, it is increased following oophorectomy (Ma, Blanchet *et al.* 2007).

The aim of this project was to investigate the mechanisms by which gender affects severity of OA.

Materials and Methods OA was induced in 10-week-old male and female C57B/6 mice by destabilization of the medial meniscus (DMM). OA was assessed by histological assessment of the joints at 4, 8 and 12 weeks post-DMM or sham surgery. Activity levels in mice were assessed using LABORAS (Laboratory Animal Behaviour Observation Registration and Analysis System), which is able to distinguish different types of activity. RNA was extracted from mouse knee joints for RT-PCR analysis 6 h following surgeries.

Results Histological analysis confirmed previous studies demonstrating an increase in severity of OA in male compared with female mice. This increase in disease was not associated with increased activity. We have recently described the molecular response to acute joint destabilization, which reveals the mechanosensitive induction of a number of inflammatory response genes and potential repair factors (Burleigh, Chanalaris *et al*. 2012). When we studied this panel of 47 genes in male and female joints, we found that some responded identically, while others were more highly expressed in the female joints. Specifically, there was no difference in the levels of Adamts5, IL1b or Mmp13, but TIMP1, inhibin, versican and Mmp3 were significantly higher in female joints.

Discussion Premenopausal female mice have significantly less severe OA compared with males, and this cannot be attributed to higher levels of activity in male mice. The response to acute joint destabilization reveals an increase in predominantly matrix, repair and anti-inflammatory genes in female mice compared with males, thereby suggesting that female mice may mount a more anti-inflammatory/repair response compared with males.

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A7

In vitro knock-down of Sox9 affects cell survival via p21 and Cyclin D1 and favours osteogenic differentiation of mesenchymal stem cells (MSC)

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Introduction Sox9 is a key transcription factor in early chondrogenesis. But there is evidence that Sox9 is also related to cell cycle regulation or tumorigenesis. Our aim is to determine the role of Sox9 in undifferentiated rat MSC for cell survival and the impact of Sox9 on osteogenic differentiation.

Materials and Methods MSC were transduced with a Sox9-specific shRNA or the Sox9 full-length cDNA sequence. The Sox9 level was determined via qRT-PCR, Western blotting and luciferase reporter assays. Appropriate markers were subsequently analysed by qRT-PCR, ELISA and Western blotting. Proliferation was quantified via cell counting and BrdU–ELISA, cell cycle distribution via FACS and apoptotic activity via caspase 3/7 ELISA. Cellular localization of cyclin D1 and p21 was determined with immunofluorescence. Osteogenic differentiation was induced for up to 21 days, marker genes were analysed via qRT-PCR, and mineralization was assessed with Alizarin red staining.

Results Sox9 silencing results in a decrease in α11 Integrin, Mmp13, Bcl-2, Vegfa, Col1a1, Runx2, p300, ALP and an increase in osteocalcin (OC) and Dlx3 mRNA expression. OC protein level increased, apoptotic activity accelerated, proliferation rate and S-phase population were reduced and p21 and cyclin D1 expression increased. Immunofluorescence revealed more nuclear p21 in Sox9 knock-down cells. Osteogenic differentiation induced an earlier up-regulation of Runx2, Mmp13, Vegfa and OC in Sox9 knock-down cells after 14 days, whereas Msx2 and Dlx5 expression was diminished.

Discussion We conclude a negative effect of Sox9 on apoptosis and a positive effect on cell proliferation and viability. An increase in p21 and cyclin D1 after Sox9 reduction points to an important role in cell survival and cell cycle control. Presumably, Sox9 regulates the nuclear export/import signal of p21, leading to a retention of p21 in the nucleus and to a reduced proliferation rate. Thereby, the involvement of kinases like GSK-3ß, and subsequent degradation of p21, needs to be considered. We furthermore suggest an acceleration of osteogenic differentiation due to a reduced Sox9 dose and a direct or indirect interaction of Sox9 with one of the major osteogenic transcription (co) factors Runx2, Msx2 or Dlx3, all containing at least one potential Sox9 binding site.

A8

The role of substance P and noradrenalin in callus differentiation

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Introduction During the progress of fracture healing, bone and fracture callus become innervated by the substance P (SP) and